strain EG10368 to chloramphenicol resistance using electroporation. Transfer of the recombinant plasmid to EG10368 was confirmed by restriction enzyme analysis of plasmid DNAs recovered from the EG10368 transformants. One chloramphenicol resistant colony was selected and designated EG12121. The *cry1C* gene in EG12121 was designated *cry1C-R148D K219A* (SEQ ID NO:60) and the encoded crystal protein designated Cry1C-R148D K219A (SEQ ID NO:61). The recombinant *cry1C* plasmid in EG12121 was designated pEG943 (FIG. 9).

Strains EG12115 (Cry1C wild-type), EG11822 (Cry1C-R148A), EG12111 (Cry1C-R148A K219A), EG11832 (Cry1C-R148D), and EG12121 (Cry1C-R148D K219A) were grown in C2 medium as described in Example 4. The spore-Cry1C crystal suspensions recovered from the spent C2 cultures were used for bioassay evaluation against neonate larvae of *Spodoptera exigua* and *Trichoplusia ni* as described in Example 4. In two sets of replicated eight-dose bioassays against *S. exigua*, the EG12111 and EG12121 Cry1C proteins were indistinguishable from the EG11822 and EG11832 Cry1C proteins, respectively. In bioassays against *T. ni*, however, further improvements in toxicity were observed for the combinatorial mutants (Tables 12 and 13).

TABLE 13

BIOASSAY EVALUATION OF THE COMBINATORIAL MUTANT CRY1C-R148A K219A AGAINST NEONATE LARVAE OF *TRICHOPLUSIA NI*

Strain	Toxin	LC_{50}[1] (95% C. I)[2]
EG12115	Cry1C	52 (32-97)
EG11822	Cry1C-R148A	24 (21-29)
EG12111	Cry1C-R148A K219A	18 (16-21)

[1] Concentration of Cry1C protein that causes 50% mortality expressed in ng crystal protein per 175 mm^2 well.

[2] 95% confidence intervals.

TABLE 14

BIOASSAY EVALUATION OF THE COMBINATORIAL MUTANT CRY1C-R148D K219A AGAINST NEONATE LARVAE OF *TRICHOPLUSIA NI*

Strain	Toxin	LC_{50}[1] (95% C. I)[2]
EG12115	Cry1C	40 (34-48)
EG11832	Cry1C-R148D	35 (29-43)
EG12121	Cry1C-R148D K219A	23 (19-28)

[1]Concentration of Cry1C protein that causes 50% mortality expressed in ng crystal protein per 175 mm^2 well.

[2]95% confidence intervals.

EXAMPLE 10 -- CRY1C-R148D COMBINATORIAL MUTANTS CONTAINING OTHER SUBSTITUTIONS IN LOOP α6-7

Additional combinatorial mutants were constructed using *cry1C-R148D K219A*, contained on pEG943, as a template for PCR™-mediated mutagenesis. A modification of the overlap extension PCR™ procedure (Horton *et al.*, 1989) was used to generate these combinatorial mutants (FIG. 10). Briefly, a PCR™ was performed using pEG943 as a template and the opposing primers H (SEQ ID NO:52) and F (SEQ ID NO:20). The amplified DNA fragment contained the R148D mutation as well as the unique *Nhe*I restriction site marking the nucleotide substitutions encoding the K219A mutation in loop α6-7. This PCR was performed using *Taq* polymerase and *Taq* Extender™ and following the protocol recommended by Stratagene. A second DNA fragment was amplified by the PCR™ using pEG943 as a template and the mutagenic oligonucleotide primer K (SEQ ID NO:63) and the opposing primer L (SEQ ID NO:64). In this instance, the PCR™ was performed using the thermostable polymerase Deep Vent™ and following the protocol recommended by New England Biolabs, Inc.

Primer K: (SEQ ID NO:63)

5′-CGGGGATTAAATAATTTACCGAAANNAACGTATCAAGATTGGATAAC-3′

N (25) = 50% C; 50% G

N (26) = 33.3% C; 33.3% G, 33.3% A

Primer L: (SEQ ID NO:64)

5′-GGATAGCACTCATCAAAGGTACC-3′

The mutagenic primer K incorporated mutations in the codon for serine (S) at position 220 of Cry1C. Six different amino acid substitutions are predicted from the mutagenesis procedure: arginine (R), alanine (A), glutamic acid (E), glutamine (Q), glycine (G), and proline (P). The mutagenic primer K also eliminates the unique *Nhe*I site in pEG943 and restores the lysine residue at position 219. Thus, *cry1C* clones incorporating this primer and containing substitutions at S220 can be distinguished from the template *cry1C-R148A K219A* gene by the loss of the *Nhe*I site.

The amplified DNA fragments were purified following agarose gel electrophoresis using the Geneclean II® procedure. To perform the overlap extension PCR™, approximately equimolar amounts of the two DNA fragments were mixed together and amplified using the flanking primers H (SEQ ID NO:52) and L (SEQ ID NO:64). Annealing of complementary strands from the two DNA fragments allows for extension from their 3′ ends (FIG. 10). Fully extended strands can then serve as templates for amplification using the flanking primers. The resulting amplified DNA fragment was purified following agarose gel electrophoresis using the Geneclean II® procedure and digested with the restriction endonucleases *Bbu*I and *Age*I. The *Bbu*I-*Age*I restriction fragment containing the 5′ portion of the *cry1C* gene was purified following agarose gel electrophoresis using the Geneclean II® procedure. In order to subclone this restriction fragment and express the mutant *cry1C* genes in *B. thuringiensis*, the *cry1C* plasmid, pEG943, (FIG. 9) was cleaved with *Bbu*I, *Nhe*I, and *Age*I, treated with calf intestinal alkaline phosphatase, and the resulting DNA fragments resolved by agarose gel electrophoresis. The vector fragment was excised from the gel and purified using the Geneclean II® procedure. The pEG943 vector fragment was subsequently ligated to the